Gene 559 (2015) 177–183

Contents lists available at ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

Tellurium as a valuable tool for studying the prokaryotic origins of mitochondria

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ARTICLE INFO

Article history:
Received 3 November 2014
Accepted 20 January 2015
Available online 30 January 2015

Keywords:
Tellurite
Saccharomyces cerevisiae
Mitochondria
Transmission electronic microscopy
Endosymbiosis
Proteobacteria

ABSTRACT

Mitochondria are eukaryotic organelles which contain the own genetic material and evolved from free-living Eubacteria, namely hydrogen-producing Alphaproteobacteria. Since 1965, biologists provided, by research at molecular level, evidence for the prokaryotic origins of mitochondria. However, determining the precise origins of mitochondria is challenging due to inherent difficulties in phylogenetically reconstructing ancient evolutionary events. The use of new tools to evidence the prokaryotic origin of mitochondria could be useful to gain an insight into the bacterial endosymbiotic event that resulted in the permanent acquisition of bacteria, from the ancestral cell, that through time were transformed into mitochondria. Electron microscopy has shown that both proteobacterial and yeast cells during their growth in the presence of increasing amount of tellurite resulted in dose-dependent blackening of the culture due to elemental tellurium (Te^0) that formed large deposits either along the proteobacterial membrane or along the yeast cell wall and mitochondria. Since the mitochondrial inner membrane composition is similar to that of proteobacterial membrane, in the present work we evidenced the black tellurium deposits on both, cell wall and mitochondria of ρ^+ and respiratory deficient ρ^- mutants of yeast. A possible role of tellurite in studying the evolutionary origins of mitochondria will be discussed.

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1. Introduction

Several experimental evidences allowed setting out the mitochondrion as the product of an ancient symbiosis in which an oxidative bacterium took up residence in the proto-nucleated cell that had developed motility and endocytosis (Gray et al., 2004; Embley and Martin, 2006; Koonin, 2010). Following this initial symbiotic event, most of the genes of the mitochondrion were transferred to the nuclear DNA (nDNA) where they now reside, are replicated and transcribed. The resultant nDNA-encoded mitochondrial genes are transcribed into mRNA, translated on cytosolic ribosome into proteins, and imported selectively into the mitochondrion. This mitochondrial protein import is frequently mediated by an amino terminal targeting peptide that is removed on entrance of the polypeptide into the mitochondrial matrix (Schatz and Butow, 1983; De Grassi et al., 2005; Schneider, 2011).

Mitochondria possess a separate autonomously replicating genome (Borst, 1972; Dujon, 1981; Wolf and Del Giudice, 1988; Bernardi, 2005), which encodes a few polypeptides that are translated by the mitochondrial protein synthesizing machinery (Schatz and Mason, 1974). Most of the mitochondrial polypeptides are encoded by the nuclear genome and synthesized on cytoplasmic ribosomes. The mitochondrial protein synthesizing system differs from that of the nucleus, but has several functional similarities to that of bacteria (Borst and Grivell, 1971; Borst, 1972). Both respond to the same antibiotics affecting different sites on the ribosomes. Initiation and elongation factors of mitochondrial protein synthesis can be replaced by homologous factors from *Escherichia coli* (Borst and Grivell, 1971).

Even though eukaryotes earlier thought to lack mitochondria have now been shown to contain some vestiges of that original

Abbreviations: ATP, adenosine triphosphate; bp, base pairs; DAPI, 4′,6-diamidino-2-phenylindole; EDTA, ethylenediaminetetraacetic acid; mRNA, messenger DNA; mtDNA, mitochondrial DNA; nDNA, nuclear DNA; PIPES, piperazine-*N*,*N*′-bis (2-ethanesulfonic acid); PMSF, phenylmethylsulfonyl fluoride; TEM, transmission electron microscopy.

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http://dx.doi.org/10.1016/j.gene.2015.01.060
0378-1119/© 2015 Published by Elsevier B.V.

endosymbiosis, either through the maintenance of hydrogenosomes, mitosomes or nuclear genes of mitochondrial origin (Gray et al., 2004; Embley and Martin, 2006), reasoning continues about the occasions of the founding endosymbiotic event (Embley and Martin, 2006; Koonin, 2010). Existing hypotheses, making use mitochondrial genome and proteome data, agree that the mitochondrial ancestor was most closely related to α-Proteobacteria (Andersson et al., 1998, 2003; Gray et al., 2001; Wu et al., 2004; Fitzpatrick et al., 2005; Esser et al., 2007; Williams et al., 2007; Ettema and Andersson, 2009). However, up to now the origin of the ancestral mitochondria within the α-Proteobacteria remains to be an unresolved question (Esser et al., 2004; Thrash et al., 2011). To find new tools for studying the prokaryotic origin of mitochondria could be a further chance to gain an insight into that origin.

Potassium tellurite (K_2TeO_3) has long been recognized as toxic to eukaryotic cell and to most microorganisms, particularly Gram-negative bacteria (Zannoni et al., 2008); furthermore, because of its antimicrobial properties, it has been used in selective media for the isolation of a number of naturally occurring tellurite-resistant bacterial species (Taylor, 1999; Zannoni et al., 2008). It has been argued that tellurite toxicity results from its ability to act as a strong oxidizing agent against a variety of cell components (Summers and Jacoby, 1977; Turner et al., 1999; Pérez et al., 2007). Microorganisms can reduce the toxicity of tellurite, either by decreasing its uptake, enhancing its efflux, or converting it into less toxic elemental tellurium. When grown in the presence of potassium tellurite, bacteria form black colonies due to the deposition of intracellular crystals of elemental tellurium (Te^0) (Taylor, 1999; Trutko et al., 2000; Lloyd, 2003; Zannoni et al., 2008).

The capacity to reduce Te is not restricted to prokaryotes, but also eukaryotes, including fungi, yeasts, plants and animal tissues that may carry out various reactions leading to form black Te^0 precipitates (Barrnett and Palade, 1957; Nagai, 1965; Corfield and Smith, 1970; Smith, 1974; Gharieb and Gadd, 1998; Ollivier et al., 2008; Zannoni et al., 2008; Massardo et al., 2009). Tucker et al. (1962) demonstrated by X-ray diffraction analysis that the black precipitate produced in bacterial cells grown in potassium tellurite medium consisted of metallic tellurium (Te^0). Later on, Nagai (1965) showed that the colonies of respiratory-deficient (petites) and respiratory-proficient *Saccharomyces* species varied in their reduction of K_2TeO_3 depending on culture conditions. Respiratory-proficient colonies turned black while petite colonies remained nearly white suggesting a potential involvement of mitochondrial function in this process.

Successively, electron microscopy examination revealed the location of metalloid deposits, either selenium (Gerrard et al., 1974) or tellurium (Taylor et al., 1988; Taylor, 1999; Trutko et al., 2000; Klonowska et al., 2005) on the inner membranes of bacteria. Moreover, the involvement of the respiratory chain, specifically by various terminal oxidases in Gram-negative bacteria, in the reduction of tellurite was demonstrated (Trutko et al., 2000; Zannoni et al., 2008). Similar evidences on the involvement of respiratory enzymes (dehydrogenases and associated enzymes) in the reduction of tellurite in a eukaryotic system (albino rats), and its exclusive precipitation within the mitochondrial inner membrane were reported (Barrnett and Palade, 1957).

In this study we preliminarily measured by 4′,6-diamidino-2-phenylindole (DAPI) staining the mitochondrial DNA content of respiration-proficient (ρ^+) and respiration-deficient (ρ^- or ρ^0) *Saccharomyces cerevisiae* strains, and then analyzed their ability to grow in the presence of K_2TeO_3. By transmission electron microscopy (TEM) we investigated the capacity of these strains to precipitate K_2TeO_3 into elemental tellurium (Te^0) on the inner mitochondrial membrane or other cell structures. This analysis was finally extended to three representative α-, β- and γ-proteobacterial strains and to an archean strain in order to gain additional evidence of the proteobacterial origin of mitochondria.

2. Materials and methods

2.1. Yeast and bacterial strains

In this study, we used a number of *S. cerevisiae* strains listed in Table 1. The yeast strains were controlled for auxotrophies and the ability to grow (for ρ^+ cells) or the inability to grow (for ρ^-, mit^-, ρ^0 cells) on glycerol medium. Bacterial strains used in this study were: *E. coli* strain C600 (Del Giudice, 1979), *Neisseria lactamica* NL4627 (Cantalupo et al., 2001), *Sphingomonas cynarae* JCM 17498^T (Talà et al., 2013) and *Halobacterium salinarum* DSM 3754^T (Minegishi et al., 2012).

2.2. Media and growth conditions for yeasts

S. cerevisiae strains were grown in: yeast-extract dextrose (YED) (yeast extract 10 g L^{-1}, glucose 20 g L^{-1}), yeast-extract glycerol (YEG) (yeast extract 10 g L^{-1}, glycerol 30 mL L^{-1}), minimal medium (YNB) (yeast Difco nitrogen base without amino acids 6.7 g L^{-1}, glucose 20 g L^{-1}) broths at 28 °C. To all solid media 20 g L^{-1} Bacto agar was added.

2.3. Media and growth conditions for bacteria

E. coli strain C600 was grown in Luria–Bertani (LB) broth at 37 °C. *N. lactamica* NL4627 was cultured in Gonococcal (GC) broth (proteose peptone no. 3 Difco 15 g L^{-1}, corn starch 1 g L^{-1}, K_2HPO_4 4 g L^{-1}, KH_2PO_4 1 g L^{-1}, NaCl 5 g L^{-1}) supplemented with 1% (v/v) Polyvitox (BioMerieux) at 37 °C in 5% CO_2. *S. cynarae* JCM 17498^T was cultivated in Tryptone Soya Broth (TSB) (Oxoid Ltd., Basingstoke, Hampshire, England) at 28 °C. *H. salinarum* DSM 3754^T was grown in liquid Medium 97 (casamino acids 7.5 g L^{-1}, yeast extract 10 g L^{-1}, Na_3 citrate 3 g L^{-1}, KCl 2 g L^{-1}, $MgSO_4 \cdot 7H_2O$ 20 g L^{-1}, $FeSO_4 \cdot 7H_2O$ 0.05 g L^{-1}, $MnSO_4 \cdot 7H_2O$ 0.2 mg L^{-1}, NaCl 250 g L^{-1}) at 37 °C. To all solid media 20 g L^{-1} Bacto agar was added when requested.

2.4. DAPI staining and epifluorescence microscopy

Staining of fixed cells was performed by 4′,6-diamidino-2-phenylindole (DAPI). Cells were fixed with 4% (v/v) glutaraldehyde for 30 min at room temperature by directly adding glutaraldehyde into the culture. After two changes with NS buffer (20 mM Tris–HCl pH 7.6, 0.25 M sucrose, 1 mM EDTA, 1 mM $MgCl_2$, 0.1 mM $ZnSO_4$, 0.1 mM $CaCl_2$, 0.8 mM PMSF, 0.05% (v/v) 2-mercaptoethanol), cells were stained with 1 μg mL^{-1} of DAPI dissolved in NS buffer on a glass slide (Williamson and Fennell, 1979; Miyakawa et al., 1994). Samples were examined under excitation by UV light and photographs taken with a Neopan 1600 film (ASA 1600, Fuji, Tokyo, Japan) with an exposure time of 12.8 s. All observations were made with a model BHS-RFK epifluorescence microscope equipped with appropriate objectives (Dplan Apo 100UVPL and 100UV; Olympus Optical Co., Ltd., Tokyo, Japan).

2.5. Growth of microorganisms in the presence of K_2TeO_3

A stock solution (4 mg mL^{-1}) of K_2TeO_3 hydrate minimum 90% (Sigma) was prepared by dissolving 44.4 mg of solute in 10 mL culture media. The solution was adjusted to pH 7–7.5 with 1 M HCl, and sterilized by filtration through a 0.22 μm pore diameter filter (Millipore). The clear stock solution was stored at 4 °C.

To evaluate the minimum inhibitory concentration (MIC) of K_2TeO_3, yeasts and bacterial strains were streaked on agarized media without or with 5 μM–1.2 mM K_2TeO_3. In particular, *S. cerevisiae* strains, *E. coli* and *S. cynarae* were cultivated, respectively, on YED, LB and TSB agar media; *N. lactamica* was grown on GC agar supplemented with 1% (v/v) Polyvitox; *H. salinarum* DSM 3754^T was cultivated on agarized Medium 97. The plates were incubated at controlled temperature for different

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